Supplemental Document

Biomedical Optics EXPRESS

Shot noise limits on binary detection in multiphoton imaging: supplement

AARON K. LAVIOLETTE AND CHRIS XU*

School of Applied and Engineering Physics, Cornell University, Ithaca NY 14853, USA
**cx10@cornell.edu*

Supplement DOI: https://doi.org/10.6084/m9.figshare.16802119

Parent Article DOI: https://doi.org/10.1364/BOE.442442

Shot Noise Limits on Binary Detection in Multiphoton Imaging: Supplemental Document

Supplementary Note 1

Here we show how Eq. (15) can be approximated using the Gaussian approximation. We consider the decision rule in Eq. (12), but we approximate p_0 and p_1 (an likewise c_0 and c_1) as Gaussian probability (and cumulative) distributions, with means $\mu_0 = B$, and $\mu_1 = S + B$, and variances, $\sigma_0^2 = B$, and $\sigma_1^2 = S + B$. We note that in this case γ is no longer restricted to an integer because the Gaussian distribution is not discrete. Thus without considering randomization for a moment, when the threshold γ is to be used to do the classification (as is the case in Eq. (6)), then one can write

$$P_F = 1 - c_0(\gamma) = 1 - \Phi\left(\frac{\gamma - \mu_0}{\sigma_0}\right), \tag{S1}$$

and

$$P_D = 1 - c_1(\gamma) = 1 - \Phi\left(\frac{\gamma - \mu_1}{\sigma_1}\right), \tag{S2}$$

where $\Phi(x) = 0.5\mathrm{erfc}\left(-x/\sqrt{2}\right)$ is the standard normal cumulative distribution function. Thus, it is possible to have arbitrary P_F and P_D without randomization, and so randomization is no longer employed (i.e., we can set q arbitrarily in Eq. (12)) [1,2]. Thus, we have that P_D and P_F will be simily given by Eqs. (S1) and (S2), and so we have that the maximum P_D given a P_F is

$$P_D(P_F) = 1 - \Phi\left[\frac{\sigma_0}{\sigma_1}\Phi^{-1}\left(1 - P_F\right) + \frac{\mu_0 - \mu_1}{\sigma_1}\right]. \tag{S3}$$

The AUC is then computed as

$$\mathrm{AUC} = \int_0^1 \mathrm{d}P_F\, P_D(P_F) = \Phi\left(\frac{\mu_1 - \mu_0}{\sqrt{\sigma_0^2 + \sigma_1^2}}\right) = \Phi\left(\frac{S}{\sqrt{S + 2B}}\right). \tag{S4}$$

The integral can be solved using Feynman's trick and results in the approximation in Eq. (16).

Supplemental Note 2

Here we show how the signal calculations in section 3.1 are performed. We assume that the signal is determined by a diffraction limited focus, thus under the paraxial approximation we have that [3]

$$S_2 \approx \frac{1}{2} g^{(2)} \phi C T \eta \sigma_2 n_0 \frac{8\langle P(t)\rangle^2}{\pi\lambda} \exp(-2z/\ell_e) \tag{S5}$$

and,

$$S_3 \approx \frac{1}{3} g^{(3)} \phi C T \eta \sigma_3 n_0 \frac{3.5(\mathrm{NA})^2 \langle P(t)\rangle^3}{\lambda^3} \exp(-3z/\ell_e) \tag{S6}$$

where $g^{(n)} = g_p^{(n)} / (f\tau)^{n-1}$ is the n[th] order temporal coherence and $g_p^{(n)}$ is a numerical parameter which is calculated based on the pulse shape. Here we have assumed that ℓ_e (typically >100 μm) is much greater than the axial resolution of the microscope (typically <10 μm).

Due to our constraint on saturation, the repetition rate is calculated as [3],

$$f = \frac{1}{\tau}\frac{\langle I_0(t)\rangle}{I_p} = \frac{1}{\tau}\frac{\pi(\mathrm{NA})^2}{I_p\lambda^2}\langle P(t)\rangle \exp(-z/\ell_e) \tag{S7}$$

where $\langle I_0(t)\rangle$ is the time-average intensity at the focus, and I_p is the peak intensity at the focus, which can be written in terms of the saturation parameter, α_{sat}, as [3],

$$I_p = \left(\frac{\alpha_{sat}}{g_p^{(n)}\sigma_n\tau}\right)^{1/n}. \tag{S8}$$

Thus, putting everything together one finds that,

$$S_2 = \frac{8}{2\pi^2}\phi C T \eta n_0 \frac{\alpha_{sat}^{1/2}\lambda\sigma_2^{1/2}\left(g_p^{(2)}\right)^{1/2}}{\tau^{1/2}(NA)^2}\langle P(t)\rangle e^{-z/\ell_e} \tag{S9}$$

and

$$S_3 = \frac{3.5}{3\pi^2}\phi C T \eta n_0 \frac{\alpha_{sat}^{2/3}\lambda\sigma_3^{1/3}\left(g_p^{(3)}\right)^{1/3}}{\tau^{2/3}(NA)^2}\langle P(t)\rangle e^{-z/\ell_e}. \tag{S10}$$

We note from Eqs. (S9) and (S10), that small changes in α_{sat} will not significantly affect the results. Since α_{sat} is raised to the 1/2 and 2/3 for 2P and 3P excitation, respectively, the ratio of S_3 to S_2 is proportional to $\alpha_{sat}^{1/6}$. Therefore, exact knowledge of α_{sat} is inconsequential for the comparison of 2P and 3P imaging quality performed in section 3.1 provided α_{sat} is of reasonable values for typical imaging experiments.

Supplemental Figures

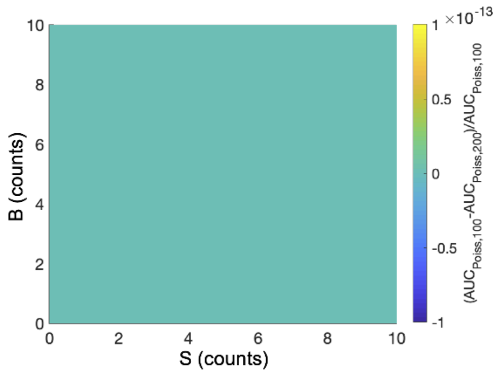

Fig. S1. Relative error between the AUC computed with 100 and 200 terms (defined as $(\mathrm{AUC}_{\mathrm{Poiss},100} - \mathrm{AUC}_{\mathrm{Poiss},200}) / \mathrm{AUC}_{\mathrm{Poiss},200}$) is shown for the range of S and B in Fig. 1. The differences are negligible.

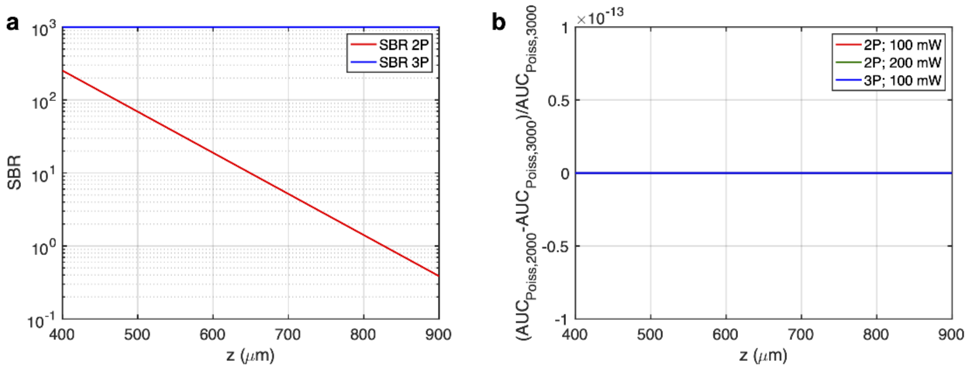

Fig. S2. (a) SBR as a function of depth used in Fig. 4. (b) Relative error between the AUC computed with 2000 and 3000 terms (defined as $(\mathrm{AUC}_{\mathrm{Poiss},2000} - \mathrm{AUC}_{\mathrm{Poiss},3000}) / \mathrm{AUC}_{\mathrm{Poiss},3000})$ is shown for the range of S and B in Fig. 4. The differences are negligible. Note also that the 2P and 3P lines are on top of each other.

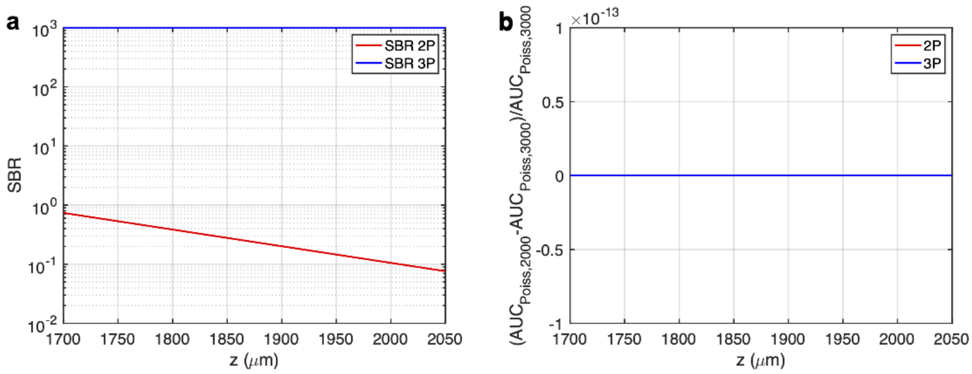

Fig. S3. (a) SBR as a function of depth used in Fig. 5. (b) Relative error between the AUC computed with 2000 and 3000 terms (defined as $(\mathrm{AUC}_{\mathrm{Poiss},2000} - \mathrm{AUC}_{\mathrm{Poiss},3000}) / \mathrm{AUC}_{\mathrm{Poiss},3000})$ is shown for the range of S and B in Fig. 5. The differences are negligible. Note also that the 2P and 3P lines are on top of each other.

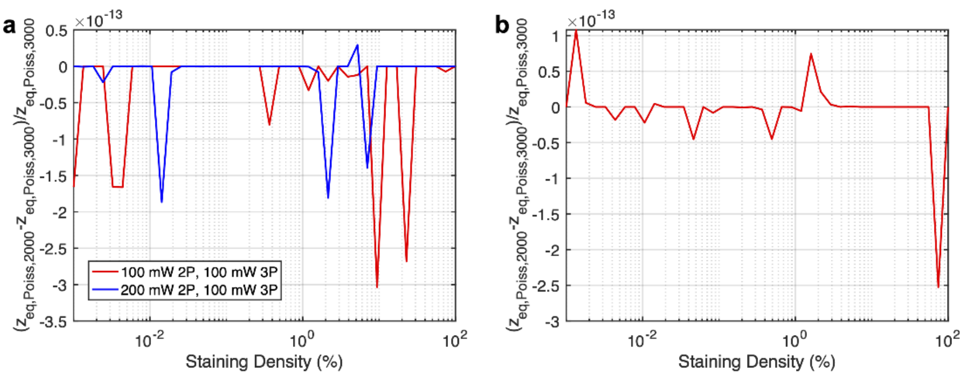

Fig. S4. Relative error between the z_{eq} computed with 2000 and 3000 terms (defined as $(z_{eq,\mathrm{Poiss},2000} - z_{eq,\mathrm{Poiss},3000}) / z_{eq,\mathrm{Poiss},3000})$ is shown for the range of staining density in (a) Fig. 6a and (b) Fig. 6b. The differences are negligible.

References

1. H. V. Poor, *An Introduction to Signal Detection and Estimation*, Second (Springer-Verlag Berlin Heidelberg, 1994), Chap. 2.
2. B. C. Levy, *Principles of Signal Detection and Paramater Estimtion* (Springer Science and Business Media LLC, 2008), Chap. 2.
3. C. Xu and W. W. Webb, "Multiphoton excitation of molecular fluorophores and nonlinear laser microscopy," in *Topics in Fluorescence Spectroscopy*, J. R. Lakowicz, ed. (Plenum Press, 2002).